Release of Soluble FcγRII/CD32 Molecules by Human Langerhans Cells: A Subtle Balance Between Shedding and Secretion?

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Freshly isolated human Langerhans cells (LC) express two forms of FcγRII: a membrane-associated form detected by monoclonal antibody (MoAb) anti-CD32, which recognize an extracytoplasmic epitope of the molecule, and a soluble secreted form, whose existence is suggested by reverse transcriptase–polymerase chain reaction (RT-PCR) experiments. Indeed, RT-PCR performed on total LC RNA reveals the presence of two FcγRIIA mRNA, one encoding the FCγRIIA with a transmembrane region (membranous form) and the other without this region (soluble form). Densitometry studies performed on the two PCR products reveal that the ratio between the membranous form and the soluble secreted form is about 1.5. LC maintained in culture for 24–48 h lose the major part of their membrane FcγRII expression (shown by flow cytometry) and release soluble FcγRII molecules (revealed by dot-blot assay), but maintain the same ratio of the two FcγRIIA mRNA. The disappearance of the membrane-associated FcγRII may be explained either by modification of its recycling pathway or by proteolytic cleavage of the receptor at the cell surface. Thus, soluble FcγRII molecules generated during LC culture may result from proteolytic cleavage of the cell-surface receptor and/or secretion of a soluble form derived from the translation of an alternate spliced mRNA. Interestingly, addition of TNF-α (10 ng/ml) to the culture medium i) maintains the expression of the membranous form, which can be detected on the LC surface at the same level as on freshly isolated LC, and ii) reverses the ratio (to 0.6) of the two FcγRII mRNA, the mRNA encoding the soluble form becoming predominant. Thus, TNF-α seems to modify the expression of the FcγRII at the mRNA level, favoring the secretion of soluble FcγRII molecules, and changes the fate of the membranous FcγRII. *J Invest Dermatol 99:15S–17S, 1992*

Three classes of receptors for the Fc fragment of IgG (FcγR) have been described on human cells: FcγRI/CD64, FcγRII/CD32, and FcγRIII/CD16 [1]. Molecular cloning of their cDNA has demonstrated that they all three belong to the immunoglobulin superfamily and are integral membrane proteins with an amino-terminal extracellular portion containing two or three immunoglobulin-related domains, a single-membrane–spanning domain, and a carboxy-terminal cytoplasmic domain of variable length. There is, however, one exception, the neutrophil FcγRIII, which was found to be anchored in the membrane via a glycosyl-phosphatidylinositol moiety. These three FcγR can be distinguished on the basis of their molecular weight, their affinity for human and mouse immunoglobulin subclasses, their reactivity with specific monoclonal antibodies (MoAb) and their cell distribution (reviewed in [2]). FcγRI is expressed only on mononuclear phagocytes, whereas FcγRII is present on a much broader range of cells, including not only mononuclear phagocytes but also granulocytes, platelets, and B lymphocytes. FcγRIII is expressed on macrophages, granulocytes, and killer/natural killer (K/NK) cells. Thus, these three FcγR are expressed on distinctive but overlapping populations of cells and various combinations can be noted. Although macrophages bear all three FcγR, monocytes and granulocytes express only two of the three FcγR: FcγRI and FcγRII in the case of monocytes, FcγRII and FcγRIII in the case of granulocytes. K/NK cells bear only FcγRIII, whereas platelets and B lymphocytes only express FcγRII.

HUMAN EPIDERMAL LANGERHANS CELLS EXPRESS ONLY THE FCγRII/CD32 CLASS OF RECEPTORS

Since the work of Stingl et al [3] in 1977, human epidermal Langerhans cells (LC) are known to possess FcγR. This characteristic was demonstrated by a rosette technique, using bovine erythrocytes sensitized by rabbit IgG anti-bovine erythrocytes. However, at this time, the molecular and functional heterogeneity of the FcγR was unknown. Recently, using anti-FcγRI, anti-FcγRII, and anti-FcγRIII MoAb, we have investigated by immunolabeling techniques and rosette assays the classes of FcγR expressed by normal human LC [4]. First evidence that LC express FcγRII came from experiments in which murine IgG1-coated erythrocytes were observed to form rosettes with LC, but not with LC that had been pre-incubated with an anti-FcγRII MoAb. Furthermore, labeling of LC with FITC-conjugated anti-FcγR MoAb and immunoprecipita-

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Abbreviations:
FcγR: receptor for the Fc fragment of IgG
LC: Langerhans cells
MoAb: monoclonal antibody

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tion of LC protein extracts with various MoAb confirmed the presence of FcγRII/CD32 and showed no detectable FcγRI and FcγRIII. However, this study also revealed that the expression of FcγRII at the LC surface was not stable. Indeed, after 24 to 48 h of culture, human LC lose their FcγRII as revealed by flow cytometry. We therefore investigated whether the disappearance of the membrane-associated FcγRII was due to a modification in its recycling pathway or resulted from a proteolytic cleavage of the receptor at the cell surface. Such proteolysis of FcγRII has been previously observed at the cell surface of mouse L cells transfected with a murine β1 FcγRII cDNA [5], resulting in the appearance of soluble forms of FcγRII in the cell-culture supernatants, corresponding to the extracellular portion of the receptor. Likewise, this mechanism seems to be also involved in the production of soluble FcγRII molecules by activated human B lymphocytes [6].

LOSS OF FcγRII/CD32 EXPRESSION BY CULTURED HUMAN LANGERHANS CELLS IS ACCOMPANIED BY THE APPEARANCE OF SOLUBLE FcγRII/CD32 MOLECULES

In order to investigate the mechanism(s) underlying the disappearance of FcγRII/CD32 from the LC surface, epidermal cell suspensions, LC-enriched, and LC-depleted human epidermal cells were cultured for 24 h, at 37°C, in serum-free medium. The membrane FcγRII/CD32 expression was evaluated at several times by double immunolabeling (CD1a and CD32) techniques, whereas the presence of soluble CD32 molecules in cell-culture supernatants was concomitantly analyzed using a direct dot-blot assay with the ^{125}I-labeled anti-CD32 MoAb, IV.3. We observed that increasing amounts of soluble FcγRII/CD32 accumulated in the cell-culture supernatants whereas the FcγRII/CD32 expression decreased at the LC surface during culture. The concentration of soluble CD32 molecules increased during the first 3 h of culture and decreased thereafter up to 24 h of culture. These soluble CD32 molecules were observed in supernatants of LC isolated by the immunomagnetic bead technique, but could not be detected in supernatants of LC-depleted epidermal cells. Thus, we concluded that soluble CD32 molecules are produced by LC and appear concomitantly with the disappearance of FcγRII/CD32 molecules from the LC surface.

The simultaneity between the disappearance of CD32 molecules from the LC surface and the appearance in the medium of soluble CD32 molecules suggested that CD32 molecules are released from LC surface by a proteolytic mechanism that affects membrane FcγRII/CD32. However, soluble FcγRII/CD32 may also be synthesized by translation of an alternatively spliced transcript of FcγRII [7] that encodes for a hydrophilic molecule containing the extracellular and intracytoplasmic domains of the FcγRII but not the transmembrane domain. Such a mRNA is likely to encode a secreted soluble form of FcγRII/CD32, although there is at present no evidence for the existence of this protein. Hence, we first investigated whether LC produce a transmembrane domain deleted FcγRII/CD32 mRNA.

HUMAN EPIDERMAL LANGERHANS CELLS PRODUCE A TRANSMEMBRANE-DOMAIN-DELETED FcγRIIA mRNA

In humans, at least three genes (named IIA, IIB, and IIC) encode FcγRII/CD32 [1]. The proteins encoded by the IIA and IIC genes differ mainly in their signal peptides; thus, the FcγRIIA and FcγRIIC are very similar. The FcγRIIB differs primarily from the two other forms by its cytoplasmic tail. Furthermore, due to multiple splicing schemes of the pre-mRNA, all these genes are likely to encode several proteins: at least two FcγRIIB (b1 and b2), which differ by the length of their cytoplasmic tail, and two FcγRIIA, which differ by the presence or absence of the transmembrane domain. However, although the FcγRIIA membrane-associated form is expressed at the cell surface, the soluble protein corresponding to the transmembrane-domain-deleted FcγRIIA has not yet been clearly identified in vivo or in vitro.

In order to detect the presence in LC of a possible transmembrane-domain-deleted FcγRIIA mRNA, total RNA of freshly isolated LC and of cultured LC was isolated by the guanidium isothiocyanate method. Amplification of reverse transcribed RNA by polymerase chain reaction (RT-PCR) was performed using two oligonucleotides specific for the extracellular and the cytoplasmic domain of the FcγRIIA, thus amplifying a fragment spanning the transmembrane domain. Two cDNA fragments of approximately 500 and 350 bp long were observed. Cloning and sequencing of these two fragments confirmed that both fragments encode for FcγRIIA and revealed that they differed by a 123-bp deletion corresponding to the transmembrane coding region (Fig 1). Thus, LC are likely to produce both the membrane-associated form and the soluble secreted form of FcγRIIA.

TNFα MODULATES THE EXPRESSION OF THE MEMBRANE ASSOCIATED FORM AND OF THE SOLUBLE SECRETED FORM OF FcγRII/CD32 IN CULTURED HUMAN LANGERHANS CELLS

In order to improve LC viability, we then added human recombinant TNFα (10 ng/ml) to epidermal cell-culture medium. In these conditions, little variation in the expression of FcγRII/CD32 on the LC surface was observed during the first 24 h of culture. However, the direct dot-blot assay with the ^{125}I-labeled MoAb anti-CD32, IV.3, showed the presence of soluble FcγRII/CD32 molecules in the culture supernatants after 1, 3, 6, and 18 h of culture. Moreover, RT-PCR performed on the TNFα-treated LC indicated that the presence of TNFα in the culture medium provoked an increase in the amount of transmembrane-deleted mRNA within the first hour of culture. This mRNA predominated over the membrane form, encoding mRNA after 3, 6, and 24 h of culture. Densitometry studies performed on the two PCR products revealed that the ratio between the mRNA encoding the membrane-associated form and the mRNA encoding the soluble-secreted form decreased from 1.5 in freshly isolated LC to 0.6 in LC cultured for 24 h in the presence of TNFα. However, this study allowed only a relative quantification between the two forms.

CONCLUSION AND PROSPECTS

In conclusion, our results show that LC express at least the FcγRIIA gene, and preliminary results from our laboratory suggest that the FcγRIIB gene is also transcribed, indicating that LC resemble monocytes and macrophages as far as FcγRII expression is concerned [1]. Two transcripts derived from the IIA gene have been observed: one mRNA encodes for a membrane-associated receptor whereas another mRNA, lacking the transmembrane-domain-encoding sequence, is likely to generate a soluble-secreted form. These two transcripts can be detected in sheets of epidermis obtained from healthy patients at ratios similar to those observed in purified fresh LC. Thus, the procedure of purification of LC does not seem to affect our results. The biosynthesis of both forms of FcγRIIA mRNA appears to be regulated by TNFα, which apparently stimulates the production of the transmembrane-deleted mRNA. Because soluble FcγRII/CD32 are released from cultured LC, our results strongly suggest that the secretion of a soluble CD32 molecule accounts for at least a part of the release of CD32 into the culture medium, whereas some of these molecules could also result from a proteolytic cleavage of the cell-surface receptor.

As shown in this review, many questions still remain unsolved. In particular, the mechanisms leading to the release by LC of soluble FcγRII/CD32 molecules have to be detailed. Also, it is unclear whether there is an effective production and secretion by LC of the transmembrane-deleted form, resulting from the alternative splicing of the primary transcript of FcγRIIA, and whether soluble forms also derive from proteolysis of the membrane receptor. The respec-

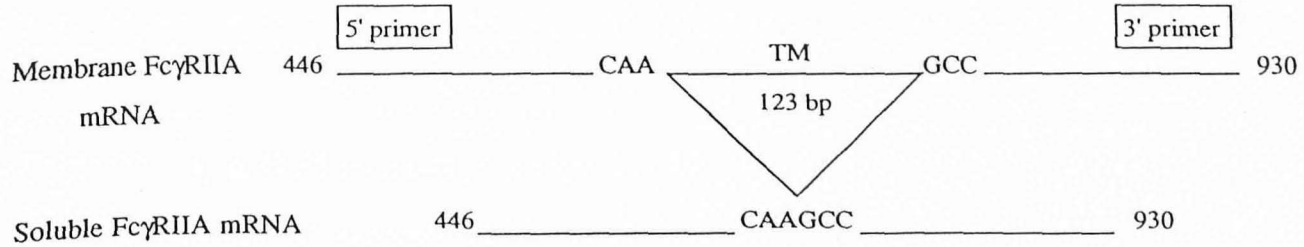

Figure 1. Two fragments of FcγRIIA are obtained by RT-PCR from total RNA of human epidermal Langerhans cells using two oligonucleotides flanking the transmembrane domain of FcγRIIA. Cloning and sequencing of these two fragments revealed that they differed by a 123-bp deletion *(open triangle)* corresponding to the transmembrane coding region. Numbering is in accordance with Brooks et al [10].

tive functions of the membrane forms of FcγRIIA and FcγRIIB remain to be defined, notably with regard to their internalization in LC by receptor-mediated endocytosis. Last, the role of the soluble CD32 molecules released by LC within the epidermis or elsewhere is totally unknown. Soluble proteins derived from membrane-associated receptors for the Fc fragment of immunoglobulins have been described for the low-affinity receptors FcγRIII/CD16 and FcεRII/CD23 and their functions are still a subject of debate [8,9]. Soluble CD32 could be involved in the regulation of LC "maturation," and the cellular processes that are dependent upon activation of membrane CD32 (on LC, as well as on other cell types), and/or may act upon other cells that migrate in inflammatory skin such as T cells, neutrophils, macrophages, and even keratinocytes.

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